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L14: Entry 1 of 1 File: DWPI [illegible]

DERWENT-ACC-NO: 2000-[illegible]
DERWENT-WEEK: 200274

TITLE: Detection and purification of substances using a Staphylococcus protein A affinity tag, useful for studying the structure, activities or interactions of proteins or for identifying potential drugs

INVENTOR: RIGAUT, G; SERAPHIN, B

PATENT-ASSIGNEE: EMBL EURO LAB MOLEKULARBIOLOGIE (EMBL-N)

PRIORITY-DATA: 1998EP-0115448 August 17, 1998

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
DE 69902746 E	October [illegible], 2002		000	C12N015/74
WO 200009716 A1	February 24, 2000	E	041	C12N015/74
AU 9957161 A	March 6, 2000		000	C12N015/74
EP 1105508 A1	June 13, 2001	E	000	C12N015/74
US 20020061513 A1	May 23, 2002		010	C12Q001/00
EP 1231276 A1	August 14, 2002	E	000	C12N015/74
JP 2002523085 W	July 23, 2002		029	C12N015/09
EP 1105508 B1	September 4, 2002	E	000	C12N015/74

DESIGNATED-STATES: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION-DATA:

PUB NO	APPL DATE	APPL NO	RELATED [illegible]
DE 699[illegible]E	August [illegible]	[illegible]	
DE 699[illegible]E	August [illegible]	1999EP [illegible]	
DE 699[illegible]E	August [illegible]	1999WO EP[illegible]	
DE 69912079E		EP 1105[illegible]	Based on
DE 699[illegible]E		WO 200[illegible]	Based on
AU [illegible]A	August [illegible]	1999WO EP[illegible]	
AU [illegible]A	August [illegible]	1999AU [illegible]	
AU [illegible]A		WO 200[illegible]	Based on
EP 1105508A1	August 17, 1999	1999EP 0944420	
EP 1105508A1	August 17, 1999	1999WO EP06022	
EP 1105508A1		WO 200009716	Based on
US2002[illegible]A1	August 17, 1999	1999WO EP06022	Cont of
US2002[illegible]A1	February 14, 2001	2001US [illegible]	
EP 1231276A1	August 17, 1999	1999EP 0944420	Div ex
EP 1231276A1	August 17, 1999	2002EP 0004765	
EP 1231276A1		EP 1105508	Div ex
JP2002522[illegible]W	August 17, 1999	1999WO EP06022	
JP2002522[illegible]W	August 17, 1999	2000JP [illegible]	
JP2002522[illegible]W		WO 200009716	Based on
EP 1105508B1	August 17, 1999	1999EP 0944420	
EP 1105508B1	August 17, 1999	1999WO EP06022	
EP 1105508B1	August 17, 1999	2002EP 0004765	Related to
EP 1105508B1		EP 1231276	Related to
EP 1105508B1		WO 200009716	Based on

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ABSTRACTED-PUB-NO: EP 1105508B
BASIC-ABSTRACT:

NOVELTY - A method for detecting or purifying substances uses polypeptides or subunits fused to at least 2 different affinity tags, at least one of which is an IgG binding domain of Staphylococcus protein A (SPA).

DETAILED DESCRIPTION - A method of detecting and/or purifying substances selected from proteins, biomolecules, complexes of proteins or biomolecules, subunits, cell components, cell organelles and cells, comprises:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding polypeptides and/or subunits of a biomolecular complex, the polypeptides or subunits being fused to at least 2 different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) maintaining the expression environment to express the polypeptides or subunits in a native form as fusion proteins with the affinity tags; and

(c) detecting and/or purifying the polypeptide or subunits by a combination of at least 2 affinity purification steps, each comprising binding the polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the polypeptides or subunits from the support material after one and another have been removed.

INDEPENDENT CLAIMS are also included for the following:

(1) a method for detecting and/or purifying [illegible]

[illegible]

(a) [illegible] at least 2 subunits [illegible] each being fused to at least one of different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) [illegible]

(c) detecting and/or purifying the complex by a combination of at least 2 different affinity purification steps each comprising binding the one or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after unbound substances have been removed;

(2) fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least 2 different affinity tags, where one of the affinity tags consists of at least one IgG binding domain of SPA;

(3) nucleic acid coding for a fusion protein of (2);

(4) a vector comprising a nucleic acid as in (3) under the control of sequences facilitating the expression of a fusion protein as in (2);

(5) a vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least 2 different affinity tags, one consisting of one or more IgG binding domains of Staphylococcus aureus protein A (SAPA), and at least one PN linker for the insertion of further nucleic acids;

(6) a vector comprising heterologous nucleic acid sequences in form of 2 or more cassettes each comprising at least one of different affinity tags, one consisting of one or more IgG binding domains of SAPA, and at least one PN linker for the insertion of further nucleic acids;

(7) a cell containing a nucleic acid of (3) or a vector of (4); and

(8) a reagent kit comprising a nucleic acid of (3) or a vector of (4), (5) or (6) for the expression of a fusion protein of (2) and support materials each capable of specifically binding one of the affinity tags.

USE - The methods can be used for the detection and/or purification of substances capable of complexing with the fusion protein (claimed). They can also be used for the detection and/or purification of cells and/or cell organelles expressing the fusion protein on their surface (claimed). They can be used for studying the structure, activities or interactions with proteins or nucleic acids. The methods not only facilitate efficient purification of proteins of interest but also allows fishing for and detecting components present in complexes with which the polypeptides or subunits are associated or complexed either directly or indirectly, e.g. molecules such as linker mediators. This would allow selective fishing, for certain substances which may be potential drugs, even from complex mixtures.

ABSTRACTED FOR [illegible]

EQUIVALENT ABSTRACTS:

NOVELTY - A method for detecting or purifying substances uses polypeptides or subunits fused to at least 2 different affinity tags (at least one of which is an IgG binding domain of Staphylococcus protein A (SPA)).

DETAILED DESCRIPTION - [illegible]

(a) providing an expression environment containing one or more heterologous nucleic acids encoding polypeptides and/or subunits of a protein complex, the polypeptides or subunits being fused to at least 2 different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) maintaining the expression environment to express the polypeptides or subunits in a native form as fusion proteins with the affinity tags; and

(c) detecting and/or purifying the polypeptides or subunits by a combination of at least 2 affinity purification steps each comprising binding the polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the polypeptides or subunits from the support material after unbound substances have been removed.

INDEPENDENT CLAIMS are also included for the following:

(1) a method for detecting and/or purifying biomolecule and/or protein complexes comprising:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding at least 2 subunits of a biomolecule complex, each being fused to at least one of different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) maintaining the expression environment to facilitate expression of the one or more subunits in a native form as fusion proteins with the affinity tags, and to allow formation of a complex between the one or more subunits and possibly other components capable of complexing with the one or more subunits; and

(c) detecting and/or purifying the complex by a combination of at least 2 different affinity purification steps each comprising binding the one or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after unbound substances have been removed;

(2) fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least 2 different affinity tags, where one of the affinity tags consists of at least one IgG binding domain of SPA;

(3) nucleic acid coding for a fusion protein of (2);

(4) a vector comprising a nucleic acid as in (3) under the control of sequences facilitating the expression of a fusion protein as in (2);

(5) a vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least 2 different affinity tags, one consisting of one or more IgG binding domains of Staphylococcus aureus protein A (SAPA), and at least one PN linker for the insertion of further nucleic acids;

(6) a vector comprising heterologous nucleic acid sequences in form of 1 or more cassettes each comprising at least one of different affinity tags, one consisting of one or more IgG binding domains of SAPA, and at least one PN linker for the insertion of further nucleic acids;

(7) a cell containing a nucleic acid of (3) or a vector of (4); and

(8) a reagent kit comprising a nucleic acid of (3) or a vector of (4), (5) or (6) for the expression of a fusion protein of (2) and support materials each capable of specifically binding one of the affinity tags.

USE - The methods can be used for the detection and/or purification of substances capable of complexing with the fusion protein (claimed). They can also be used for the detection and/or purification of cells and/or cell organelles expressing the fusion protein on their surface (claimed). They can be used for studying the structure, activities or interactions with proteins or nucleic acids. The method is not only

facilitate efficient purification of proteins of interest but also allow fishing for and detecting compounds present in complexes with which the polypeptides or subunits are associated or complexed either directly or indirectly, e.g. mediated such as linker mediated. This would allow selective fishing, for certain substances which may be potential drugs, even from complex mixtures.

NOVELTY - A method for detecting or purifying substances uses polypeptides or subunits fused to at least 2 different affinity tags at least one of which is an IgG binding domain of Staphylococcus protein A (SPA).

DETAILED DESCRIPTION - A method of detecting and/or purifying substances selected from proteins, biomolecular complexes of proteins or biomolecular subunits, cell components, cell organelles and cells, comprises:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding polypeptides and/or subunits of a biomolecule complex, the polypeptides or subunits being fused to at least 2 different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) maintaining the expression environment to express the polypeptides or subunits in a native form as fusion proteins with the affinity tags; and

(c) detecting and/or purifying the polypeptides or subunits by a combination of at least 2 affinity purification steps, each comprising binding the polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the polypeptides or subunits from the support material after unbound substances have been removed.

INDEPENDENT CLAIMS are also included for the following:

(1) a method for detecting and/or purifying biomolecule and/or protein complexes comprising:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding at least 1 subunits of a biomolecule complex, each being fused to at least one of different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) maintaining the expression environment to facilitate expression of the one or more subunits in a native form as fusion proteins with the affinity tags, and to allow formation of a complex between the one or more subunits and possibly other components capable of complexing with the one or more subunits; and

(c) detecting and/or purifying the complex by a combination of at least 2 different affinity purification steps each comprising binding the one or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after unbound substances have been removed;

(2) fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least 2 different affinity tags where one of the affinity tags consists of at least one IgG binding domain of SPA;

(3) nucleic acid coding for a fusion protein of (2);

(4) a vector comprising a nucleic acid as in (3) under the control of sequences facilitating the expression of a fusion protein as in (2);

(5) a vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least 2 different affinity tags, one consisting of one or more IgG binding domains of Staphylococcus aureus protein A (SPA) and at least one [illegible] linker for the insertion of [illegible] fusion protein [illegible];

(6) a vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least one of different affinity tags [illegible]

[illegible] binding [illegible] DNA [illegible] for the insertion of further nucleic acids;

(7) a cell containing a nucleic acid of (3) or a vector of (4); and

(8) a reagent kit comprising a nucleic acid of (3) or a vector of (4), (5) or (6) for the expression of a fusion protein of (1) and supply of material(s) each capable of specifically binding one of the affinity tags.

USE - The methods can be used for the detection and/or purification of substances capable of complexing with the fusion protein (claimed). They can also be used for the detection and/or purification of cells and/or cell organelles expressing the fusion protein on their surface (claimed). They can be used for studying the structure, activities or interactions with proteins or nucleic acids. The methods not only facilitate efficient purification of proteins of interest but also allows fishing for and detecting components present in complexes with which the polypeptides or subunits are associated or complexed either directly or indirectly, e.g. molecules such as linker mediators. This would allow selective fishing, for certain substances which may be potential drugs, even from complex mixtures.

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